Gramella portivictoriae sp. nov., a novel member of the family *Flavobacteriaceae* isolated from marine sediment

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A yellow-pigmented, Gram-negative, slowly gliding, rod-shaped, strictly aerobic bacterium (UST040801-001^T) was isolated from marine sediment. The DNA G+C content was 39·9 mol%. The predominant fatty acids were a15 : 0, i15 : 0, i15 : 0 3-OH, i17 : 1ω9c, i17 : 0 3-OH and summed feature 3, comprising i15 : 0 2-OH and/or 16 : 1ω7c (altogether representing 76·2 % of the total). MK-6 was the only respiratory quinone. Flexirubin-type pigments were not produced. Phylogenetic analysis based on 16S rRNA gene sequences indicated that *Gramella echinicola* KMM 6050^T (the only species in the genus) was the closest relative of UST040801-001^T, sharing 98·0 % sequence similarity. The DNA–DNA relatedness between UST040801-001^T and *Gramella echinicola* KMM 6050^T was 13 %. Strain UST040801-001^T can be distinguished from *G. echinicola* by means of 11 phenotypic traits. The results of molecular and phenotypic analyses suggested that UST040801-001^T represents a novel species of *Gramella*. The name *Gramella portivictoriae* sp. nov. is proposed for this bacterium, with UST040801-001^T (=NRRL 41137^T=JCM 13192^T) as the type strain.

The genus *Gramella*, belonging to the family *Flavobacteriaceae* (Bernardet *et al.*, 1996), was created recently by Nedashkovskaya *et al.* (2005) and currently consists of a single species, *Gramella echinicola*, originating from the sea urchin *Strongylocentrotus intermedius* in the Sea of Japan. In this study, we propose a novel species of *Gramella* originating from marine sediment.

During the characterization of a microbial community in marine sediment collected from Victoria Harbour, Hong Kong, the bacterial strain UST040801-001^T was isolated on an agar medium composed of 5 g peptone l^{-1}, 3 g yeast extract l^{-1} (both Oxoid) and 0·22-μm-filtered sea water (referred to as marine agar hereafter). After 48 h incubation at 30 °C on marine agar, UST040801-001^T appeared as yellow, convex, circular colonies (2–4 mm in diameter) with smooth surfaces and entire translucent margins. No diffusible pigment was observed. Unless otherwise specified, all characteristics described hereafter were based on cultures grown on marine agar for 48 h at 30 °C.

The nearly-complete 16S rRNA gene sequence of UST040801-001^T (1468 bp) was obtained using a dye terminator method, as described elsewhere (Lau *et al.*, 2004). Fragments of DNA sequence obtained from individual primers with at least six replicates were each assembled using the SEQUENCHER software package (Gene Codes). Comparison of the nearly-complete 16S rRNA gene sequence of UST040801-001^T with those available from GenBank revealed that UST040801-001^T was a member of the family *Flavobacteriaceae* and was closely related to *G. echinicola* KMM 6050^T (98·0 % sequence similarity) and to the uncharacterized marine bacteria KT0803 and KMM 6048 (97·8 and 98·4 % sequence similarity, respectively). A neighbour-joining phylogenetic tree constructed using the ARB software package (Ludwig *et al.*, 2004) showed that the four bacteria formed a distinctive clade (Fig. 1). Within this clade, UST040801-001^T formed a branch with the marine bacterium KMM 6048 without significant bootstrap support. Trees based on maximum-parsimony and maximum-likelihood methods in the ARB software package showed identical topology (Fig. 1). The level of DNA–DNA relatedness between UST040801-001^T and *G. echinicola* KMM

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST040801-001^T is DQ002871.

An electron micrograph of cells of strain UST040801-001^T, the results of API 20E, API 20NE and API ZYM tests and details of the carbon sources tested are available as supplementary material in IJSEM Online.

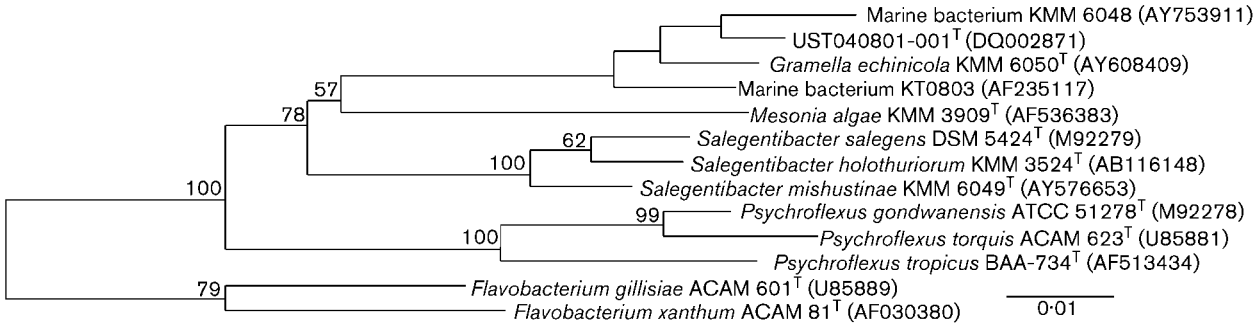

Fig. 1. Neighbour-joining tree showing the estimated phylogenetic relationships among UST040801-001^T and related species, on the basis of 16S rRNA gene sequences. Strains belonging to the genus *Flavobacterium* served as outgroups. The topologies of the trees constructed using maximum-parsimony and maximum-likelihood methods were identical to the topology in the neighbour-joining tree (not shown). Bootstrap values above 50 % (500 replicates) are indicated at nodes. GenBank accession numbers are shown in parentheses. Bar, 1 nt substitution per 100 nt.

6050^T was 13 %, which clearly indicated that UST040801-001^T should belong to a novel species of the genus *Gramella*. DNA–DNA hybridization experiments were performed by the BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent (Gent, Belgium) using photobiotin-labelled probes as described by Ezaki *et al.* (1989).

The DNA G+C content of UST040801-001^T, determined using an HPLC method (Mesbah *et al.*, 1989), was 39·9±0·2 mol% (three replicates). This value was similar to that for *G. echinicola* KMM 6050^T (39·6 mol%). The dominant cellular fatty acids of UST040801-001^T were a15 : 0, i15 : 0, i15 : 0 3-OH, i17 : 1ω9*c*, i17 : 0 3-OH and summed feature 3, comprising i15 : 0 2-OH and/or 16 : 1ω7*c* (altogether representing 76·2 % of the total), as determined using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's protocol (Table 1). MK-6 was the only respiratory quinone, as determined using an HPLC method according to Collins (1994). Menaquinones extracted from *Cellulophaga lytica* (Johansen *et al.*, 1999) and *Pedobacter heparinus* (Steyn *et al.*, 1998) served as references for MK-6 and MK-7, respectively.

Anaerobic growth was examined using the Oxoid Anaerobic System. The requirement for NaCl was tested in a medium containing (l^{-1}) 5 g $MgCl_2$, 2 g $MgSO_4$, 0·5 g $CaCl_2$, 1 g KCl, 5 g peptone and various amounts of NaCl, adjusted to pH 7·5 using KOH (Isnansetyo & Kamei, 2003). Cell morphology was examined using scanning electron microscopy (7600F; JEOL) according to Neu *et al.* (2001) (see Supplementary Fig. S1 available in IJSEM Online). The reaction to Gram stain was determined using light microscopy according to Smibert & Krieg (1994). Gliding motility was determined using phase-contrast light microscopy (Olympia) after growth on quarter-strength marine 2216 medium (Oxoid) solidified with 1 % agar according to Bowman (2000). Susceptibility to antibiotics was tested as described by Acar (1980). Flexirubin-type pigment production and carboxymethylcellulose hydrolysis were determined as described by Bernardet *et al.* (2002). Casein

Table 1. Cellular fatty acid profiles of UST040801-001^T and *G. echinicola* KMM 6050^T

Values are percentages of total fatty acids. Data for UST040801-001^T are means±SD (three replicates). Data for *G. echinicola* KMM 6050^T are from Nedashkovskaya *et al.* (2005). Strain UST040801-001^T was grown in marine agar at 30 °C for 48 h, whereas *G. echinicola* KMM 6050^T was grown in marine agar 2216 at 25 °C for 48 h.

Fatty acid	UST040801-001^T	*Gramella echinicola* KMM 6050^T
i13 : 0	0·8±0·1	–
i14 : 0	0·5±0·1	1·4
15 : 0	–	7·1
15 : 1ω6*c*	1·8±0·3	1·9
a15 : 0	6·2±0·7	7·6
i15 : 0	38·1±0·5	14·4
i15 : 1	–	1·2
i16 : 0	1·9±0·4	13·1
i16 : 1	–	5·8
i16 : 1 H	0·9±0·1	–
15 : 0 2-OH	1·0±0·2	2·0
15 : 0 3-OH	2·5±0·1	–
i15 : 0 3-OH	6·2±0·3	1·3
i16 : 0 3-OH	3·4±0·3	5·9
17 : 0 2-OH	1·5±0·2	2·6
17 : 1ω6*c*	1·7±0·3	3·6
a17 : 1ω9*c*	–	2·0
i17 : 1ω9*c*	5·7±1·3	3·5
i17 : 0 3-OH	11·2±0·3	6·7
18 : 1ω5*c*	1·0±0·1	–
Summed feature 3*	8·8±1·2	11·4
Unknown	6·3±1·6	4·6

*Comprises i15 : 0 2-OH and/or 16 : 1ω7*c*.

hydrolysis was determined as described by Norris *et al.* (1985); Tween 20, 40 and 80 hydrolysis and chitin were tested as described by Baumann & Baumann (1988). Spore formation, oxidase and catalase activities and the hydrolysis of agar, DNA and starch were tested as described by Smibert & Krieg (1994). Other enzymic activities, the substrate-utilization profile, nitrate reduction and the production of H_2S, indole and acetoin were tested using the commercial systems API 20E, API 20NE, API 50 CH and API ZYM (all from bioMérieux) and MicroLog 3 (Biolog). Cells for inoculation to the API systems were suspended in a sterile solution of sea-water mix at 22 ‰ salinity (MacDonell *et al.*, 1982). Growth on D-arabinose, D-galactose, D-glucose, glycerol, D-mannitol, D-melibiose, D-sorbitol, starch and sucrose as sole carbon sources was additionally tested using a medium that contained 0·2 g $NaNO_3$ l^{-1}, 0·2 g NH_4Cl l^{-1}, 0·05 g yeast extract l^{-1} and 4 % (w/v) carbon source in a solution of sea-water mix at 35 ‰ salinity (Nedashkovskaya *et al.*, 2003). The phenotypic characteristics of UST040801-001^T are given in the species description (and in Supplementary Table S1, available in IJSEM Online).

Strain UST040801-001^T can be distinguished from *G. echinicola* by (i) the absence of β-galactosidase activity; (ii) the ability to hydrolyse Tween 20, to produce acetoin and to utilize D-mannitol and D-sorbitol; and (iii) the inability to grow in 15 % NaCl and to hydrolyse casein and DNA (Table 2). The results of molecular analysis, together with the phenotypic characteristics, suggest that strain UST040801-001^T represents a novel species of the genus *Gramella*.

Description of *Gramella portivictoriae* sp. nov.

Gramella portivictoriae (por′ti.vic.to′ri.ae. L. n. *portus* harbour; L. gen. fem. n. *victoriae* of Victoria; N.L. gen. fem. n. *portivictoriae* from/of Victoria Harbour, Hong Kong, the source of isolation of the type strain).

Cells are Gram-negative, rod-shaped (0·6–3·6 μm), glide slowly and do not form spores. Colonies on marine agar are yellow in colour without diffusible pigment, circular, 2·0–4·0 mm in diameter and convex with smooth surfaces and entire translucent margins after 48 h at 30·0 °C. Growth is strictly aerobic and occurs between 4·0 and 36·0 °C (28·0–30·0 °C optimum), at pH 6·0–10·0 (pH 7·0–8·0 optimum) and at 1·0–6·0 % NaCl. The DNA G+C content is 39·9 mol%. The predominant cellular fatty acids are a15 : 0, i15 : 0, i15 : 0 3-OH, i17 : 1ω9*c*, i17 : 0 3-OH and summed feature 3, comprising i15 : 0 2-OH and/or 16 : 1ω7*c* (altogether representing 76·2 % of the total). The major quinone is MK-6. Flexirubin-type pigments are not produced. Susceptible to ampicillin, chloramphenicol, penicillin and tetracycline, but not to kanamycin or streptomycin. Acetoin is produced, but indole and H_2S are not. Citrate is not utilized. Nitrate is not reduced. Aesculin ferric citrate, gelatin, starch and Tweens 20, 40 and 80 are hydrolysed, but agar, casein, carboxymethylcellulose, chitin and DNA are not hydrolysed. Positive for acid phosphatase, alkaline phosphatase, α-chymotrypsin, catalase, cystine arylamidase, leucine arylamidase, valine arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), oxidase, α-galactosidase, α-glucosidase, β-glucosidase, trypsin and naphthol-AS-BI-phosphohydrolase activities. Negative for *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-fucosidase, β-galactosidase, β-glucuronidase, α-mannosidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease activities. Utilization of D-arabinose, D-galactose, D-glucose, glycerol, D-mannitol, D-melibiose, D-sorbitol, starch and sucrose as sole carbon sources is observed on agar medium supplemented with 4 % (w/v) carbon source. Utilization of γ-hydroxybutyric acid is observed with the MicroLog 3 system. However, no growth or acid production is observed from the carbon sources in the API 50 CH, API 20E and API 20NE test systems (lists of carbon sources included in the API and MicroLog 3 test systems are available in Supplementary Tables S2 and S3, respectively, in IJSEM Online.)

The type strain is UST040801-001^T (=NRRL 41137^T=JCM 13192^T), isolated from sediment in Victoria Harbour, Hong Kong.

Table 2. Differentiation of UST040801-001^T from *G. echinicola* KMM 6050^T

Data for *G. echinicola* KMM 6050^T are from Nedashkovskaya *et al.* (2005).

Characteristic	UST040801-001^T	*G. echinicola* KMM 6050^T
Growth in 15 % NaCl	−	+
Hydrolysis of:		
Casein	−	+
DNA	−	+
Tween 20	+	−
β-Galactosidase activity	−	+
Production of acetoin	+	−
Utilization of:		
D-Mannitol	+	−
D-Sorbitol	+	−

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